PerkinElmer CS Autoplex Analysis Software User Manual

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Introduction

Chapter 1

Chapter Summary

1.1 Overview

The PerkinElmer CS Autoplex Analysis software analyzes Luminex xMAP output data for various high-volume life science and diagnostic applications. For each type of application, the PerkinElmer CS Autoplex Analysis provides a separate software plug-in, each with an easy-to-use wizard for configuring an analysis.

The first supported application is ratiometric calculations for gene expression, using Luminex Output.csv files. The PerkinElmer CS Autoplex Analysis computes the ratios for samples in wells and displays the results in a spreadsheet table, scatter plot, bar chart, and distribution plot.

1.2 Starting the Software

To start the PerkinElmer CS Autoplex Analysis software

- Double click the PerkinElmer CS Autoplex Analysis Software icon on the computer screen desktop.

Note: If the icon does not appear on the desktop, click **Start** on the Windows task bar, then select **Programs, PerkinElmer, CS Autoplex Analysis Software, CS Autoplex Analysis Software.**

The following are also available from the **Start** menu for the PerkinElmer CS Autoplex Analysis Software:

- CS Autoplex Analysis Software Help
- CS Autoplex Analysis Software User Manual
- Uninstall PerkinElmer CS Autoplex Analysis Software

1.3 The CS Autoplex Analysis Software Window

The PerkinElmer CS Autoplex Analysis Software opens to a *main window*, shown in Figure 1-1. The main window is the starting place for each analysis. From here you can open and view the input file, view file header information, start an analysis, and view and save analysis results.

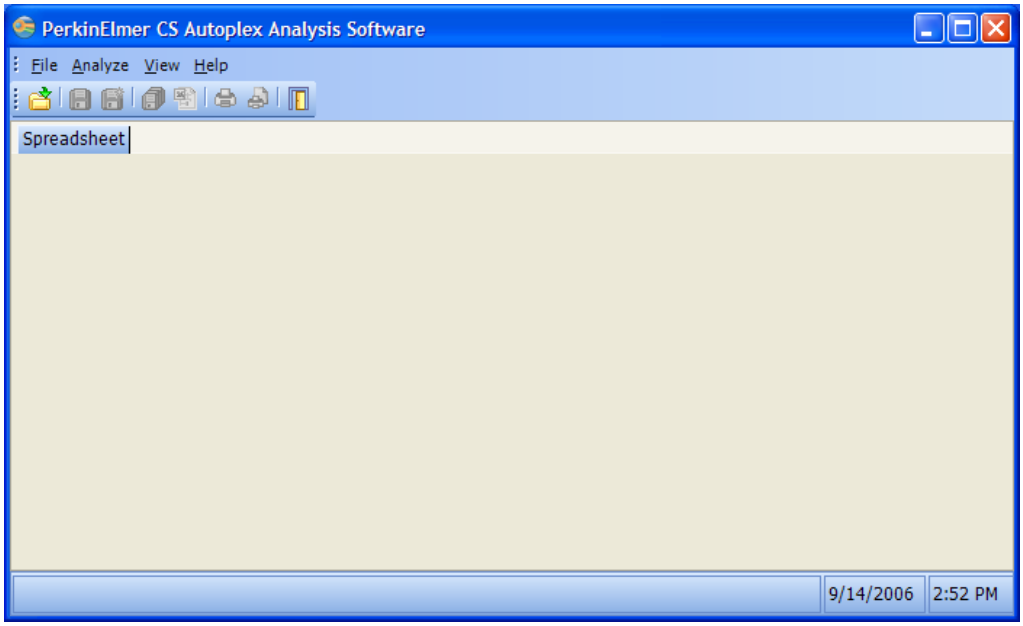
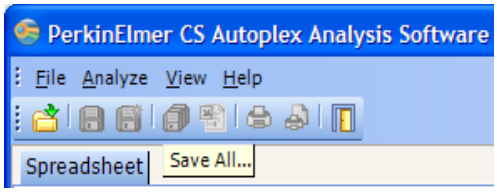


Figure 1–1 The PerkinElmer CS Autoplex Analysis Software Main Window

The main window includes the following menus and toolbar:

- **File** - a standard Windows menu to open, save, print, and export files, and exit the PerkinElmer CS Autoplex Analysis Software.
- **Analyze** - to start the wizard for a selected analysis.
- **View** - to configure the view for the current display (a spreadsheet or plot), and turn on/turn off the display for the toolbar or status bar.
- **Help** - to access online help and version information about the PerkinElmer CS Autoplex Analysis.
- **Toolbar buttons** provide a shortcut for each task on the **File** menu: Open, File, Save, Save as, Save all, Print, Print Preview, and Exit. A button for Export to Excel displays when a spreadsheet is open. Hold the mouse cursor over a button to display the button name.



1.4 Getting Help

Help for using the software is available in these ways:

- The PDF file for this user manual is available from the Windows **Start** menu by selecting **Programs**, **PerkinElmer, CS Autoplex Analysis Software, CS Autoplex Analysis Software User Manual**.
- Online help can be accessed from within the PerkinElmer CS Autoplex Analysis Software from the **Help** menu, or by pressing the **F1** key to get help for the current window.

  The on-line help is also available from the Start menu by selecting **Programs**, **PerkinElmer**, **CS Autoplex Analysis Software, CS Autoplex Analysis Software Help**.

1.4.1 Using the On-Line Help

To use the on-line help

1. From the **Help** menu, select one of the following:

- **Contents** - to browse the table of contents. Click the book icons to display lists of subtopics.
- **Index** - if you know exactly what you're looking for, type the word, or scroll through the list.
- **Search** - type a word or phrase to search, then click **List Topics**. Double-click a topic in the search results list to display it.

2. To get help for the current task:

- Press the **F1** key while in any window of the PerkinElmer CS Autoplex Analysis Software to open the help topic for that window, or access any part of the on-line help.

1.4.2 Contacting Technical Support

To contact technical support:

- Web Site: http://www.perkinelmer.com
- Telephone: 800-762-4000 (US and Canada) or (+1) 203-925-4602
- E-mail: info@perkinelmer.com
- Fax: (+1) 203-944-4904

Worldwide:
Techsupport@perkinelmer.com
Techsupport.europe@perkinelmer.com

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Running a Ratiometric Analysis for Gene Expression

Chapter 2

Chapter Summary

2.1 Overview

The Ratiometric Analysis for Gene Expression calculates the ratio for samples in all well locations. One location, or a location with replicates, is selected as a reference. Then, the ratio of an analyte in each sample well is compared to the same analyte in the specified reference location(s). Blank subtraction and normalization can also be used. See ***How the Analysis Software Handles Data*** for information.

2.2 Input Data for the Analysis

Input data for a Gene Expression Ratiometric Analysis must be an Output.csv file from a Luminex 100 instrument. Configure the assay data for export to the Output.csv using the *Data Export* tab in the *Luminex 100 IS Software Options* window. Refer to the *Luminex IS Software Manual for Version V2.3* for information on exporting data. The PerkinElmer CS Autoplex Analysis Software supports any data selected for export under the *Additional Export Stats* on the *Data Export* tab.

The following default options are recommended for exporting data:

- **Auto Export Batches**
- **Plate Location**, or **Both** under *Export Location Label Style.*
  Note that if **Sequential** is selected, the plate map view is not available in the configuration wizard (see ***Figure 2–5***), nor in the scatter plot and bar chart results after the analysis. Additionally, a distribution plot is not created for the results.

2.3 How the Analysis Software Handles Data

This section describes how the PerkinElmer CS Autoplex Analysis software handles input data for the analysis.

- Reference replicates, blank subtraction, and normalization are supported. These are described below.
- If reference replicates are used, the mean of the reference replicates is used by the software as the denominator for analysis.
- Outliers are not removed.

Prior to calculating the ratio during analysis, the CS Autoplex Analysis software checks the numerator (sample value) and denominator (the reference value) and uses one of the following:

- If either numerator or denominator value is less than one (<1), the analysis uses the value of 1, as follows:
  - If the numerator is less than one (< 1), the ratio will be *1/denominator.*
  - If the denominator is less than one (< 1), the ratio is the *numerator/1*.
- If both the numerator and denominator are less than one (<1), the ratio will be *1/1*.

During analysis, the software uses the following data:

Data Options	Data used for Ratiometic Calculations
• No blank subtraction, and • No normalization	Raw data (median, trimmed mean)
• With Blank subtraction, and • No Normalization	Blank subtracted data (blank subtracted median, blank subtracted trimmed mean)
• No Blank subtraction, and • With Normalization	Normalized data (normalized median, normalized trimmed mean)
• With Blank subtraction and • With Normalization	Normalized blank data (normalized blank-subtracted median, normalized blank-subtracted trimmed mean)

2.3.1 Reference Replicates

Reference replicates are multiple beads incubated with the same sample, and assigned the same experiment name or bead ID in the Luminex assay. If replicates are selected, the PerkinElmer CS Autoplex Analysis software uses the mean of the reference replicates as a denominator for the analysis.

2.3.2 Blank Subtraction

Blank subtraction uses one or more wells containing sample with zero content to provide a control for non-specific signals. The measured content of the blank(s) is subtracted from the measured data in each sample, in the following way:

- If a sample is identified as a blank, the software takes the median value of each bead ID in the blank sample well and subtracts it from the median and trimmed mean values of that bead ID in all other wells. If replicate blank wells are used, the value subtracted is the mean of the medians in the blank samples.

2.3.2.1 Specifying Blank Samples

To specify one or more blank samples, each blank sample must be assigned a name starting with the word “Blank” when exporting the data to an Output.csv file. For example, a blank sample could be named simply “Blank” or multiple blank samples could be named as “Blank2, “Blank3,” etc.

You may enter this information manually in the Luminex IS software or by using a patient list in the Luminex IS software. Refer to the *Luminex IS Software Manual for Version V2.3* for details for entering Sample ID information.

2.3.3 Normalization

Normalization, if enabled, corrects the intensity of each signal with respect to the control(s). The PerkinElmer CS Autoplex Analysis software uses Normalize to Total (to all beads), unless one or more internal and/or external controls have been specified, in which case the analysis software normalizes to the specified control(s). If both internal and external controls have been defined, both are used for calculating the normalization factors.

The Ratiometic Analysis software calculates the normalization using the following method:

1. The software calculates the mean of the median values, from all wells, for each bead region identified as a control (which could be all beads).

2. Within each well, the software calculates the ratio of the median value for each bead to it's corresponding mean value determined in step one.

3. Within each well, the software calculates the mean of the ratios determined in step two to determine the normalization factor for the well.

4. The normalized value is the median or trimmed mean value for each bead in a well divided by the well normalization factor determined in step three.

If blank subtraction was performed, blank subtracted values are used in place of raw values in each of the four steps.

2.3.3.1 Specifying the Internal or External Controls

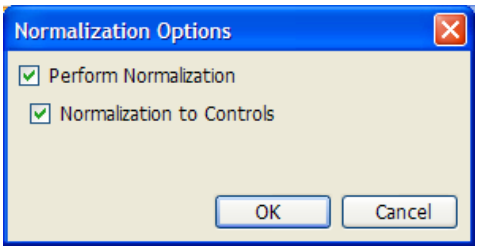
Control beads are indicated by assigning a name beginning with “IC” or “EC” to the experiment name (bead ID) when creating the Output.csv export file after the Luminex assay. Refer to the *Luminex 100 IS Developer Workbench Guide V2.3* for information on adding test names.

2.3.3.2 Enabling Normalization

Normalization is off by default, and must be enabled by checking the box in the *Normalization Options* dialog box. Once selected, the Normalization Options remain in effect until changed.

To specify normalization for an analysis

1. On the **View** menu, select **Normalization Options**.



2. In the *Normalization Options* dialog box, check the box for **Perform Normalization** to have the data normalized, or uncheck the box to skip normalization.

3. Check the box for **Normalization to Controls** if internal or external controls have been included in the assay, specified in the data by a name beginning with "IC" or "EC."

   If **Perform Normalization** is checked, but **Normalization to Controls** is not checked, the analysis uses Normalize to Total.

   If both **Perform Normalization** and **Normalization to Controls** are checked, but no controls are defined in the data, the analysis uses Normalize to Total.

2.4 Viewing the Input Data

Opening a valid input data file automatically opens the analysis wizard. To view the input data without running an analysis, cancel the wizard as described in the following procedure.

To open an input data file

1. On the **File** menu, click **Open**.

2. In the *Open* dialog box, navigate to the directory where the input data resides.

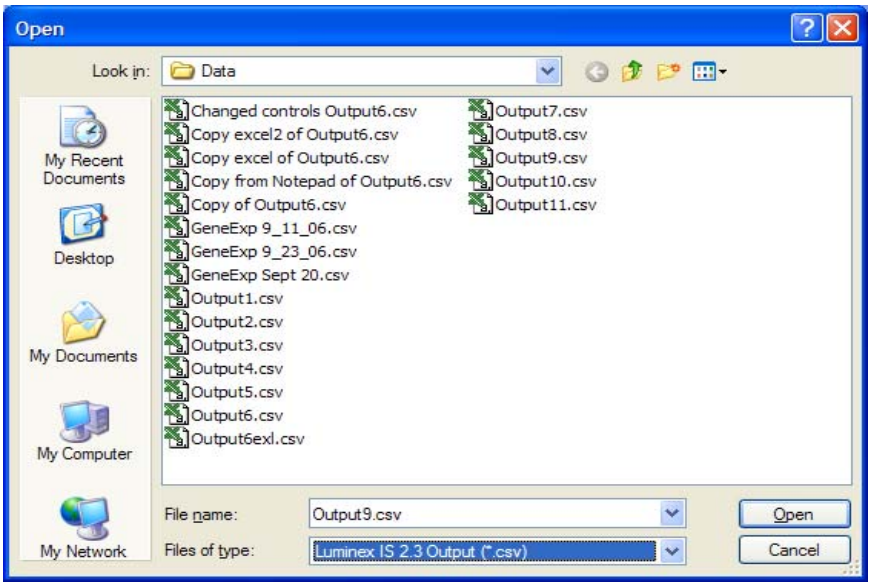


Figure 2–1 Opening a File for Ratiometric Analysis

3. In the file list, double-click the file you want to open, or type the name in the **File name** field. In the **Files of type** field, select the type of file, *Luminex IS 2.3 Output (.csv)*.

4. Click **Open.** This opens the data file and automatically opens the analysis wizard, shown in Figure 2-2.

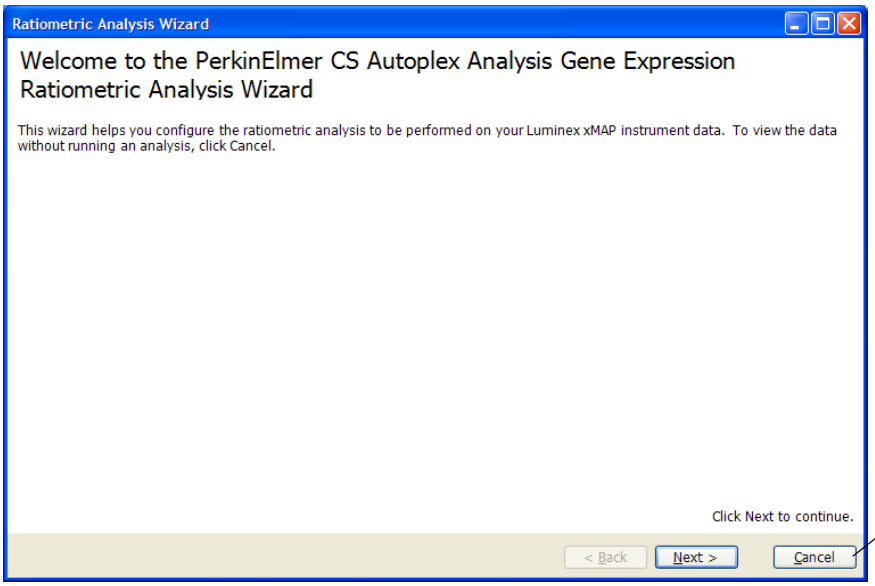


Figure 2–2 Cancelling the Analysis Wizard

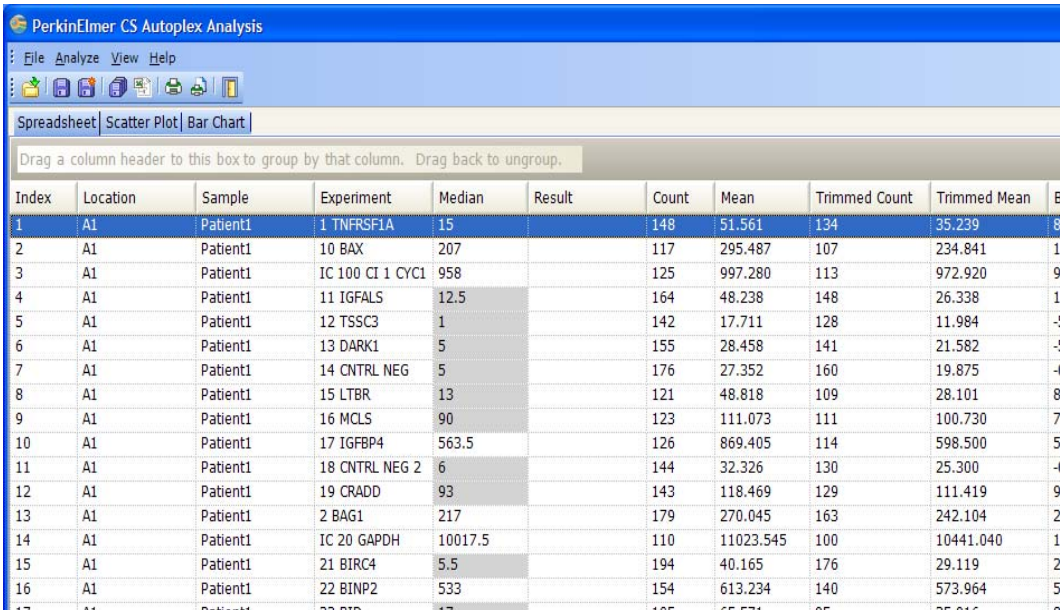
5. Click **Cancel** to view the input data without running an analysis. The raw data displays in a spreadsheet table (Figure 2-3).

Note: To start an analysis after viewing the data, restart the wizard: on the **Analyze** menu, select **Ratiometric Analysis for Gene Expression** to re-open the wizard.

If you have already run an analysis on the current raw data, the PerkinElmer CS Autoplex Analysis prompts you to save the results, and opens the wizard for an analysis on the existing data.

Data can be sorted in ascending or descending order for any column by clicking the column header, or grouped by columns, for easier viewing. See ***The Spreadsheet Table*** section, later in this chapter.

In addition, any columns in the spreadsheet can be hidden or displayed, and their order re-arranged using the *Configure Columns* dialog box. See ***Showing or Hiding Columns***.



Index	Location	Sample	Experiment	Median	Result	Count	Mean	Trimmed Count	Trimmed Mean
1	A1	Patient1	1 TNFRSF1A	15		148	51.561	134	35.239
2	A1	Patient1	10 BAX	207		117	295.487	107	234.841
3	A1	Patient1	IC 100 CI 1 CYC1	958		125	997.280	113	972.920
4	A1	Patient1	11 IGFALS	12.5		164	48.238	148	26.338
5	A1	Patient1	12 TSSC3	1		142	17.711	128	11.984
6	A1	Patient1	13 DARK1	5		155	28.458	141	21.582
7	A1	Patient1	14 CNTRL NEG	5		176	27.352	160	19.875
8	A1	Patient1	15 LTBR	13		121	48.818	109	28.101
9	A1	Patient1	16 MCLS	90		123	111.073	111	100.730
10	A1	Patient1	17 IGFBP4	563.5		126	869.405	114	598.500
11	A1	Patient1	18 CNTRL NEG 2	6		144	32.326	130	25.300
12	A1	Patient1	19 CRADD	93		143	118.469	129	111.419
13	A1	Patient1	2 BAG1	217		179	270.045	163	242.104
14	A1	Patient1	IC 20 GAPDH	10017.5		110	11023.545	100	10441.040
15	A1	Patient1	21 BIRC4	5.5		194	40.165	176	29.119
16	A1	Patient1	22 BINP2	533		154	613.234	140	573.964

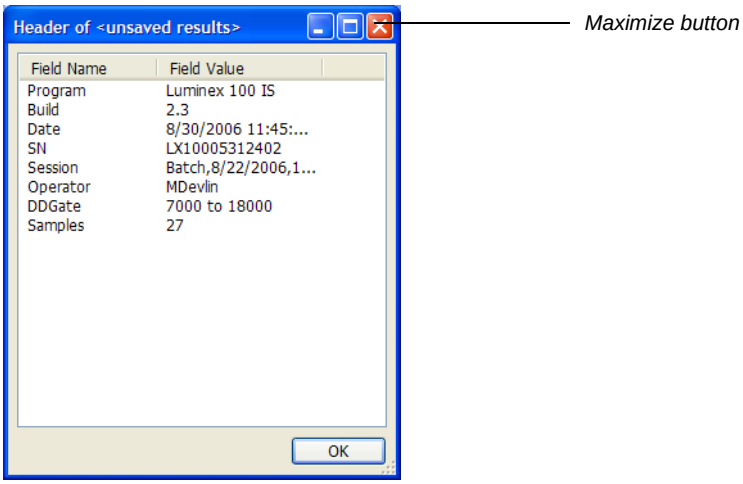
Figure 2–3 Input Data for an Analysis

2.4.1 Viewing the File Header Information

The file header includes information such as the program used, and the date that the input data was created, and by whom. This information does not display in the spreadsheet table, but is always available for viewing.

To view the file header information

1. On the **View** menu, click **Spreadsheet Options**, **Show Header.** The *Header of....* dialog box opens to display header information for the currently open file.



2. To see the full pathname for the data file, click the maximize button, or resize the dialog box by dragging the bottom right corner.

2.5 Running a Ratiometric Analysis

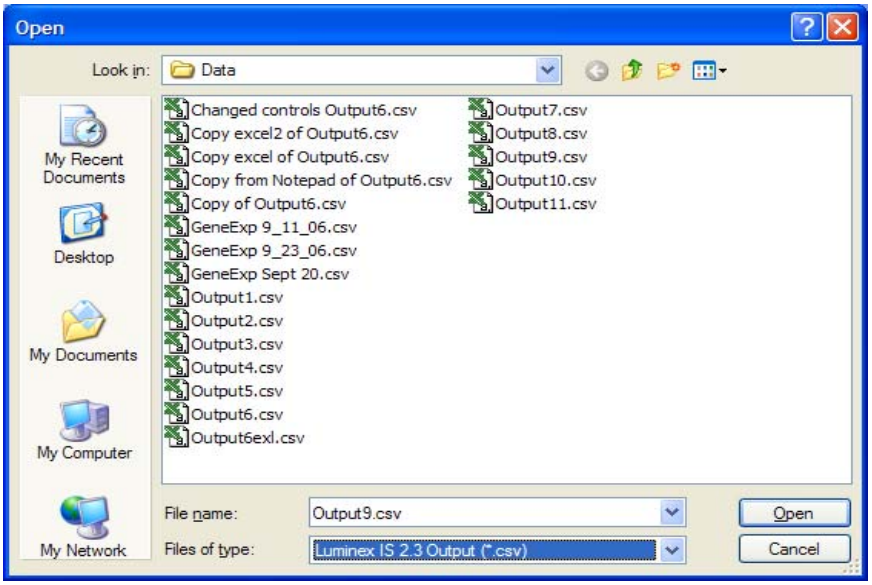
Use the Gene Expression Ratiometric Analysis wizard to configure the settings for the analysis. The wizard opens automatically when the correct type of input file is opened.

Note: If you already opened the input file and cancelled the wizard to view the data, use the **Analyze** menu to start the wizard. Select **Analyze, Ratiometric Analysis for Gene Expression**.

To run a Gene Expression Ratiometric Analysis

1. On the **File** menu, click **Open**.

2. In the *Open* dialog box, navigate to the directory where the input data resides.



3. In the file list, double-click the file that you want to open, or type the name in the **File name** field and click **Open**. This opens the data file and automatically opens the analysis wizard.

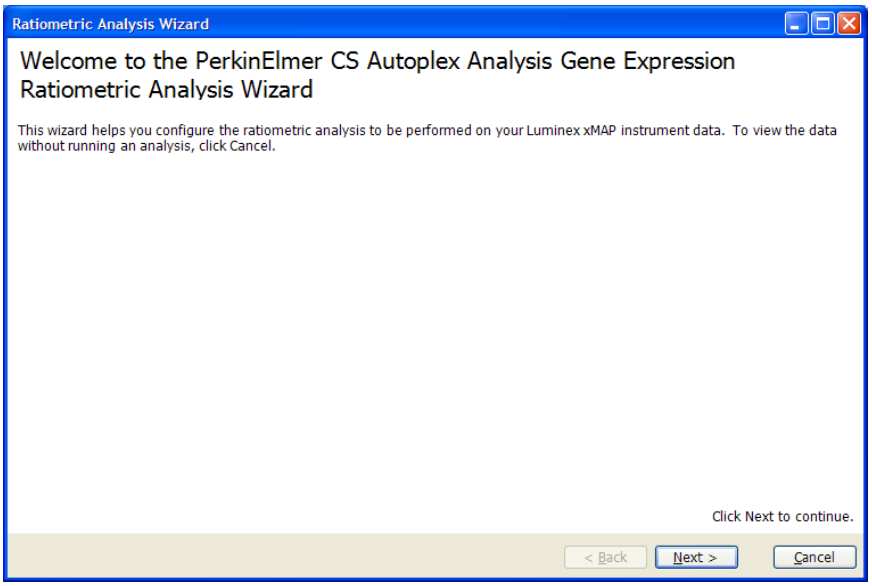


Figure 2–4 The Gene Expression Ratiometric Analysis Wizard

4. Click **Next** to continue.

5. In the *Select Reference Location(s)* window, select the location(s) to use for the ratio. To select replicates, hold down the **Ctrl** key as you click each location.

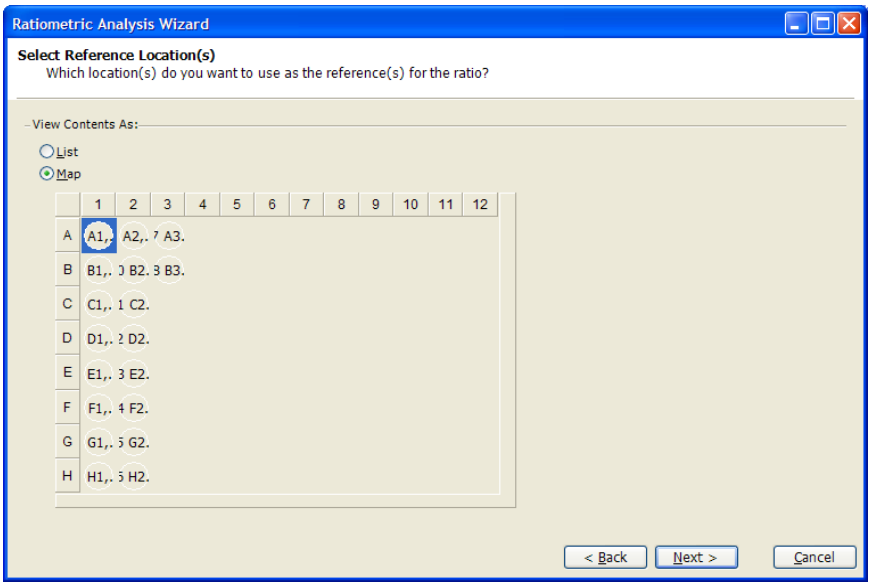
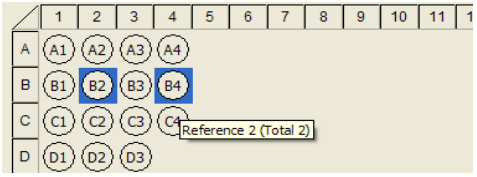


Figure 2–5 Select Reference Location(s) - Map View

Note: The map view, shown in Figure 2-5, is available only if **Plate Location** or **Both** was selected under the *Export Location Label Style* on the Luminex IS in the data export options. If **Sequential** was selected, the contents will be available only as the list, shown in Figure 2-6.

Note: Tool tips are available for the plate wells in the map view. Hold the mouse cursor over a plate well to see the sample text, if it exists, or the well information (for example A1). If you don't see the tool tips, you may need to resize the window first.



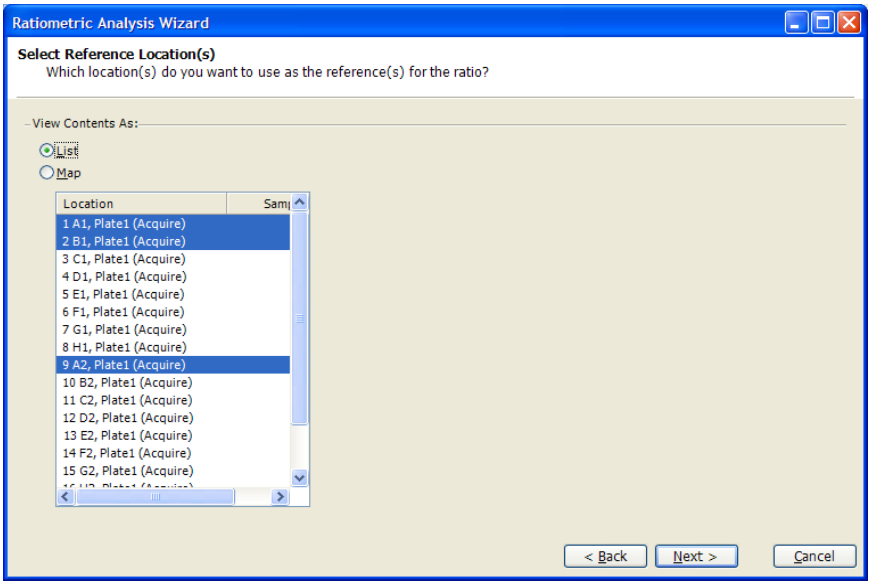


Figure 2–6 Select Reference Location(s) - List View

6. After selecting the location(s), click **Next**.

7. In the *Summary* window, review your selections.

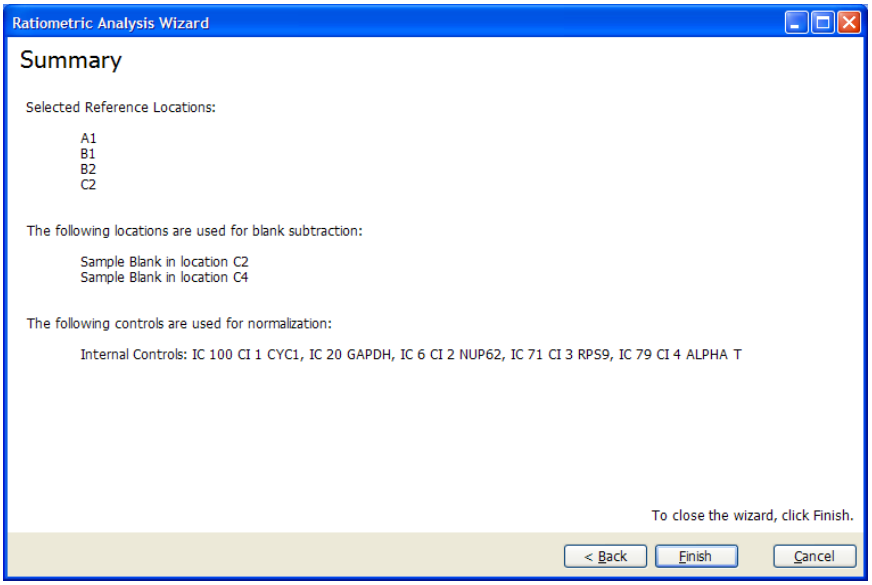


Figure 2–7 Summary Window

- To change any of the selected location(s), click **Back** once to return to the *Select Reference Location(s)* window.

- To change normalization selections, you must cancel the analysis, set the **Normalization Options** from the **View** menu, then run the analysis from the **Analyze** menu.

Note: If existing blank wells were not identified during the acquisition, the information is not available in the input file for the analysis. The blanks information can be added to the input data from within the PerkinElmer CS Autoplex Analysis software using the spreadsheet table.

Cancel the analysis and edit the data. See ***Adding Blanks or Controls Information after Acquisition***.

8. If you're satisfied with the selections, click **Finish** to start the analysis.

2.5.1 Adding Blanks or Controls Information after Acquisition

There are two ways to add the blanks information to the raw data file: by editing the spreadsheet from within the PerkinElmer CS Autoplex Analysis software (see below), or by editing the data .csv file using Microsoft Notepad (see Appendix B).

To edit the spreadsheet for blanks

1. Open the input data file and cancel the analysis wizard.

2. Group the rows by location by dragging the *Location* column header into the group box above the spreadsheet. See ***To group the data by columns*** on page 2- 12.

3. Expand each group that corresponds to the blank locations.

4. Click any cell in the *Sample* column. This puts the cell in edit mode.

5. Modify the text in the cell so that it begins with the word "Blank."

6. Click in the next row for the *Sample* column to automatically update all rows for this sample.

Note: Information for internal or external controls can be added in a similar same way, by editing the spreadsheet, and modifying the appropriate Experiment names to begin with "IC" or "EC".

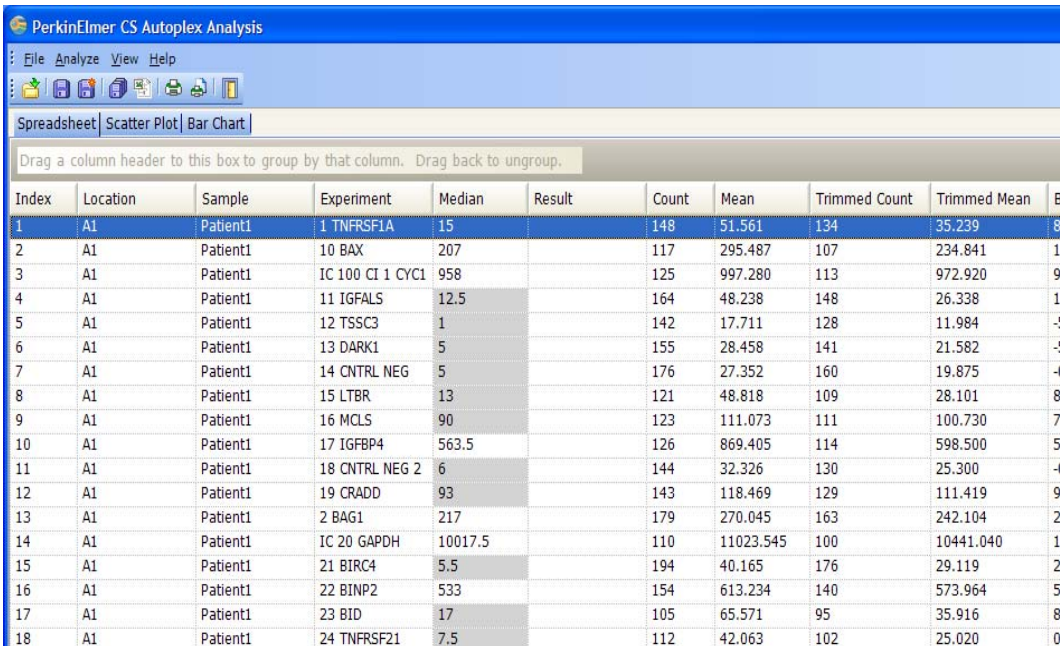
The data is now ready for analysis. From the **Analyze** menu, select **Ratiometric Analysis for Gene Expression** to open the wizard for analysis.

2.6 Reviewing the Analysis Results

The analysis results are displayed in a spreadsheet table in the PerkinElmer CS Autoplex Analysis main window. Graphical views of the data, including a scatter plot, bar chart, and distribution plot, are also provided in tabbed windows behind the spreadsheet. To bring any window to the front, click the tab name.

2.6.1 The Spreadsheet Table

The *Spreadsheet* shows the analysis results for all analytes in all locations. The customizable view lets you group and sort by column, choose which columns to display, and flag median and bead count values that fall within a specified range. Figure 2-8 is an illustration of a typical results spreadsheet, with the default columns showing.



Index	Location	Sample	Experiment	Median	Result	Count	Mean	Trimmed Count	Trimmed Mean
1	A1	Patient1	1 TNFRSF1A	15		148	51.561	134	35.239
2	A1	Patient1	10 BAX	207		117	295.487	107	234.841
3	A1	Patient1	IC 100 CI 1 CYC1	958		125	997.280	113	972.920
4	A1	Patient1	11 IGFALS	12.5		164	48.238	148	26.338
5	A1	Patient1	12 TSSC3	1		142	17.711	128	11.984
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9	A1	Patient1	16 MCLS	90		123	111.073	111	100.730
10	A1	Patient1	17 IGFBP4	563.5		126	869.405	114	598.500
11	A1	Patient1	18 CNTRL NEG 2	6		144	32.326	130	25.300
12	A1	Patient1	19 CRADD	93		143	118.469	129	111.419
13	A1	Patient1	2 BAG1	217		179	270.045	163	242.104
14	A1	Patient1	IC 20 GAPDH	10017.5		110	11023.545	100	10441.040
15	A1	Patient1	21 BIRC4	5.5		194	40.165	176	29.119
16	A1	Patient1	22 BINP2	533		154	613.234	140	573.964
17	A1	Patient1	23 BID	17		105	65.571	95	35.916
18	A1	Patient1	24 TNFRSF21	7.5		112	42.063	102	25.020

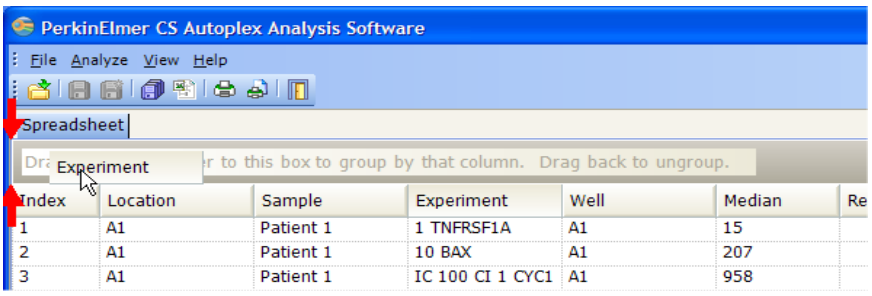
Figure 2–8 Analysis Results Spreadsheet

The displayed data columns depend both on the information that was included in the input file, and whether any columns are hidden. All data that was included in the input file is available for the results. If the data was included, but a column isn't visible, it may be hidden. See ***Display Options for the Spreadsheet*** on page 2- 15.

The information can also be grouped by a column to more easily see related data.

To group the data by columns

1. Click the column heading, for example *Experiment*, and drag it into the box above the column headings. When the red arrows display above the column headings, release the mouse button.



Index	Location	Sample	Experiment	Well	Median	Re
1	A1	Patient 1	1 TNFRSF1A	A1	15	
2	A1	Patient 1	10 BAX	A1	207	
3	A1	Patient 1	IC 100 CI 1 CYC1	A1	958	

2. The data is grouped by the column and "collapsed." The example in Figure 2-9 shows the data grouped by *Experiment*.

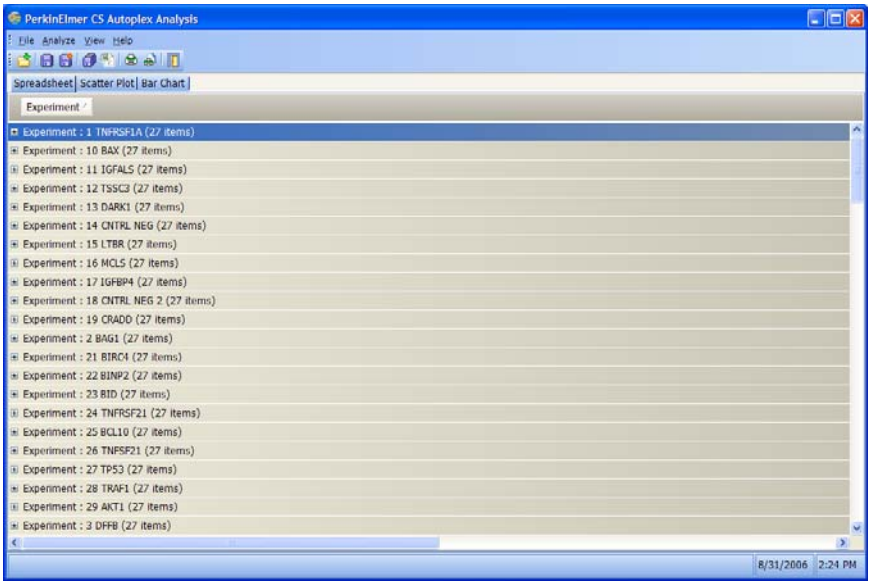


Figure 2–9 Data Columns Grouped by Experiment

3. To see all information for a grouped column, click the plus sign (+) to expand the selection. Click again to collapse it. For example, an Experiment, when expanded, shows all locations for that experiment.

4. To return to the full display of the columns, drag the column heading back down to the spreadsheet headings row.

2.6.1.1 Information Provided in the Spreadsheet Table

The following information is included in the spreadsheet for analysis results:

- Median is always included, as well as the calculated Ratio of Median, or its equivalent, after the analysis.
- Ratio of Trimmed Mean, or its equivalent, is included in the spreadsheet after analysis only if the trimmed mean data was included in the input file.
- If replicates are selected, the denominator is the mean of the replicates.
- Outliers are not removed.

Additional columns are included, depending on the input data. Refer to Table 2-1 for a description of the possible data columns. If some columns aren't visible in your spreadsheet, they may be turned off in the *Configure Columns* window, or the data may not have been included in the input file.

Table 2-1: Spreadsheet Table Columns

Item	Description
Input Data	The following columns for input data are available:
Location	Location of the sample in terms of the command list: **sequence** (1, 2, 3, etc.) **plate location** (A1, B1, C1, etc.) **both** (1 (A1), 2 (B1), 3 (C1), etc.). Or **advanced batch** (1 A1, Plate1 (Acquire), (2 B1, Plate1 (Acquire), etc.).
Sample	The name of the sample as defined in the batch setup in the Luminex 100 IS analyzer. The name is limited to 30 characters.
Experiment	This is the test name from the Output.csv file. This name defaults to the bead ID, but it can be any name entered when setting up the assay on the Luminex instrument.
Count	The number of data points in the distribution (n). The number of gated events that fell within the test's specified region.
Median	The middle value in the distribution of data.
Additional Export Stats	Columns for any of the following information are included after analysis, if the data was included with the input data (exported to the Luminex Output.csv file)
%CV	(Optional) The measure of relative dispersion within the distribution. %CV = 100 x Std Dev / Mean
Peak	(Optional) The value that is equal to the largest number of data points within the distribution. For example, in data set {1,2,2,3,3,4,5) 3 is the peak because it occurs the most number of times in the distribution list.
Trimmed Count	(Optional) The number of data points in the trimmed distribution (N_t).
Trimmed Mean	(Optional) The sum of the data points in the trimmed distribution divided by the number of data points. Trimmed Mean = $\Sigma X_i/N_t$
Trimmed %CV	(Optional) The measure of relative dispersion within the trimmed distribution. Trimmed %CV = 100 x Trimmed Std Dev / Trimmed Mean
Trimmed Peak	(Optional) The value that is equal to the largest number of data points within the trimmed distribution.
Trimmed Std Dev	(Optional) The measure of dispersion within the trimmed distribution. Trimmed Std Dev = $((N_t \Sigma X_i^2 - \Sigma X_i)^2) / N_t (N_t-1)^{1/2}$
Avg Results	(Optional) The average of any replicate samples' final test results based on a qualitative or quantitative analysis.

Item	Description
Analysis Results	After analysis, the following columns are added to the spreadsheet, and are available in the *Configure Columns* dialog box. Note: *Blank subtracted, normalized blank subtracted, or normalized data is used for the input column*.
Ratio of *<input column>* to *<well* where: • the ***<input column>*** is one of the following: Median or Trimmed Mean, and • the ***<well>*** is the first reference well that was selected for the ratio in the wizard. For example, *Ratio of Median to C1* Or **Ratio of *<input column>* to *<well>* and *replicates***	where: • If there are replicates, the column title includes “and replicates”; the replicates are not specified by name. For example, *Ratio of Median to C1 and replicates*

2.6.1.2 Display Options for the Spreadsheet

Display options for the spreadsheet include hiding columns and flagging data that fits within a specified range.

To configure the spreadsheet options

1. On the **View** menu, select **Spreadsheet Options**
   or,
   right-click anywhere on the spreadsheet to open the options menu.

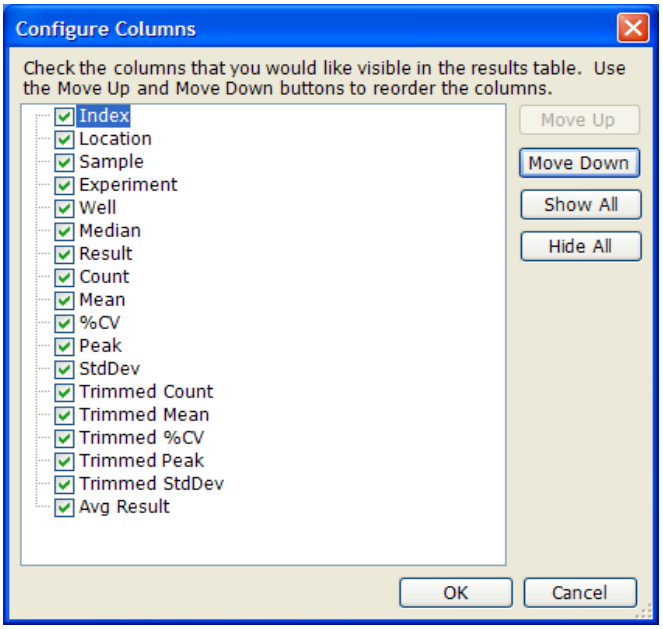
2. From the menu, select the option you want to change. The options are described in the following table.

Menu	Description
Show/Hide Columns	Select data columns to hide or display, and arrange them in order.See ***Showing or Hiding Columns*** on page 2- 16.
Show Header	View information for the current data file.See ***Viewing the File Header Information*** on page 2- 6.
Flagging Options	Specify to flag median values and bead count values that fall above or below specified values. See ***Flagging Median and Bead Count Values*** on page 2- 17.

2.6.1.3 Showing or Hiding Columns

To configure the spreadsheet columns

1. On the **View** menu, click **Spreadsheet Options**, **Show/Hide Columns**. The *Configure Columns* dialog box opens. The list of columns in this dialog box depends on the data exported to the Luminex Output.csv file. The following example shows all of the possible columns.



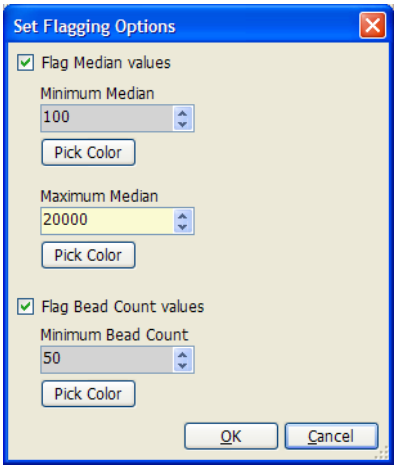
2. Check the box next to each column that you want to display in the spreadsheet. Uncheck the box next to each column that you want to hide.
   Click **Show All** to check all boxes, or check **Hide All** to uncheck all boxes.

To re-arrange the order of the columns, highlight (select) any column that you want to move, and click **Move Up** or **Move Down** to move the column to the desired position.

2.6.1.4 Flagging Median and Bead Count Values

To set the flagging options

1. On the **View** menu, click **Spreadsheet Options**, **Flagging Options**. The *Set Flagging Options* dialog box opens. The example below shows the default settings.



2. Set the options, and click **OK**. The following table describes the options.

Item	Description
Flag Median Values	Check this box to flag the median values with a background color in the spreadsheet. **Minimum Median Value** - leave the default, or specify a new value. **Pick Color** - click to open the *Color* dialog box and select a different color. Click a color and click **OK**. **Maximum Median Value** - leave the default, or specify a new value. **Pick Color** - click to select a different color for flagging.
Flag Bead Count Values	Check this box to flag the bead count values that are above the specified value. **Minimum Bead Count** - leave the default, or specify a new value. **Pick Color** - click to select a different color for flagging.

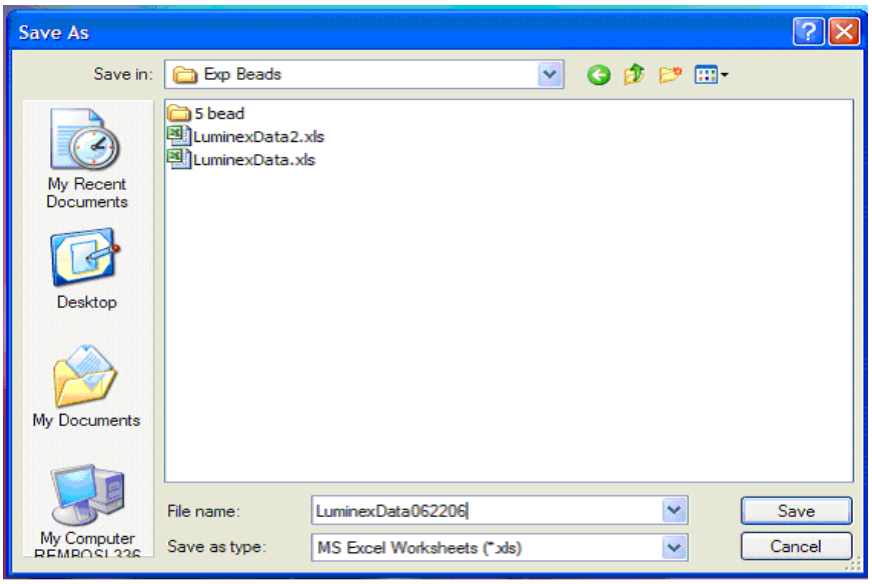
2.6.1.5 Exporting the Spreadsheet to Excel

To export the spreadsheet table to Excel

1. On the **File** menu, select **Export to Excel,** or click the Toolbar icon.

2. In the *Save as* dialog box, enter a name in the **File name** field.

3. In the **Save as type** field, select MS Excel Worksheet (*xls), and click **Save**.



2.6.2 The Scatter Plot

Data in a scatter plot shows the values for all analytes in a single well. The configurable plot lets you select a location to view, and any available column to plot. The most meaningful data to display would be the data which was used for calculating the resulting ratios. The x-axis on the bottom displays the selected column data for the reference location. If reference replicates were selected, the first reference location is displayed on the x-axis. The y-axis on the left displays the selected column data for all analytes in the selected location. See Figure 2-10 for an example of a scatter plot.

The title of the plot displays the name of the currently viewed well location, or a title that's been assigned under the Scatter Plot Options described in this section.

The plot can be printed or saved. See ***Saving the Analysis Results***.

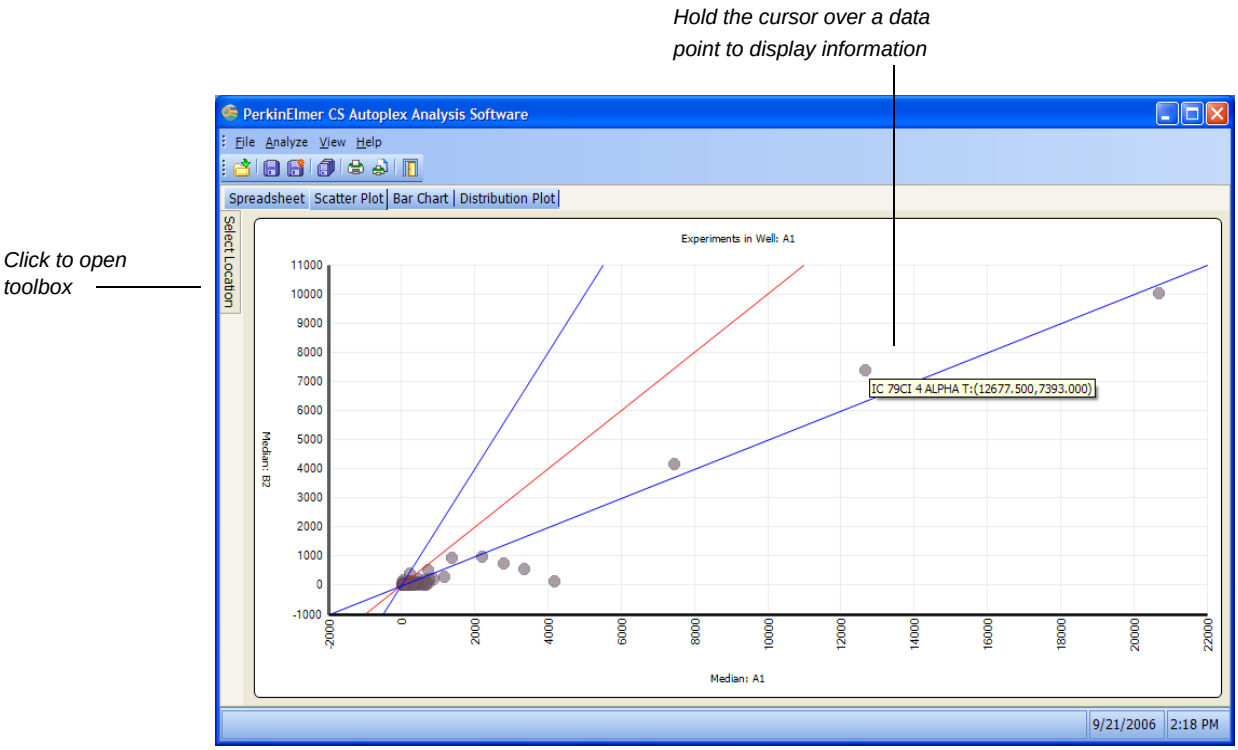


Figure 2–10 Example of a Scatter Plot

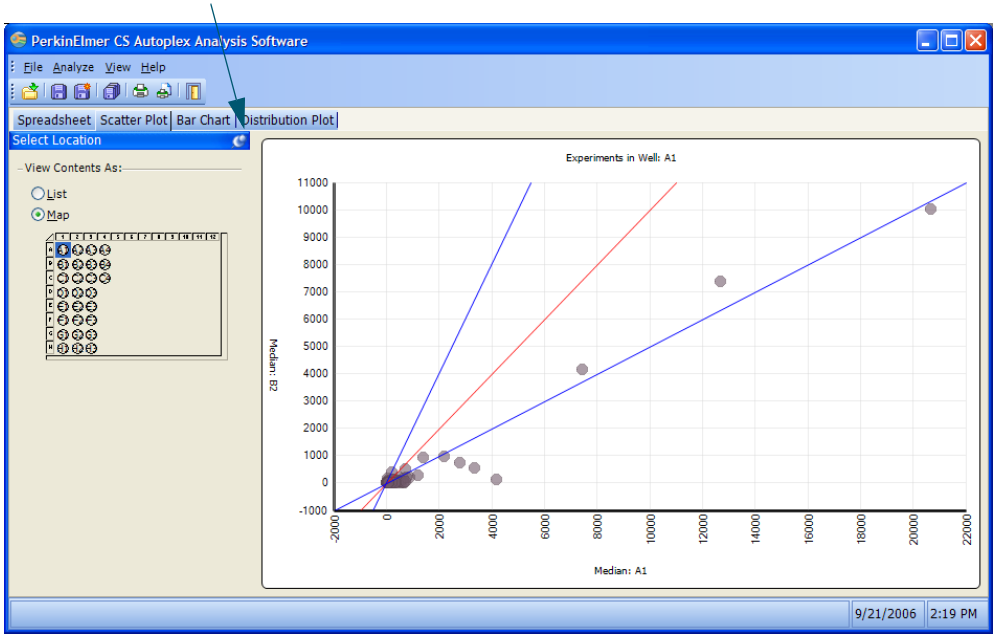
2.6.2.1 Selecting a Location to View

The PerkinElmer CS Autoplex Analysis provides a toolbox for changing the scatter plot view by location, and menu options for configuring the display (for example, changing which data column to plot or changing the range of one or both axes.)

To change the scatter plot view

1. Click **Select Location** on the left-hand side of the scatter plot. The *Select Location* toolbox opens over the plot.

Click the pushpin to keep the toolbox open and display the entire plot at the same time



2. Select a location to view, using the list or map.

   The toolbox automatically auto-hides when you click anywhere on the plot.

Note: To keep the toolbox open and display the entire plot and toolbox side by side, click the pushpin on the toolbox. Click the pushpin again to return to Auto Hide.

2.6.2.2 Display Options for the Scatter Plot

To configure the scatter plot options

1. On the **View** menu, select **Scatter Plot Options**
   Or,
   right-click anywhere on the scatter plot to open the options menu.

2. From the menu, select the option you want to change. The options are described in the following table.

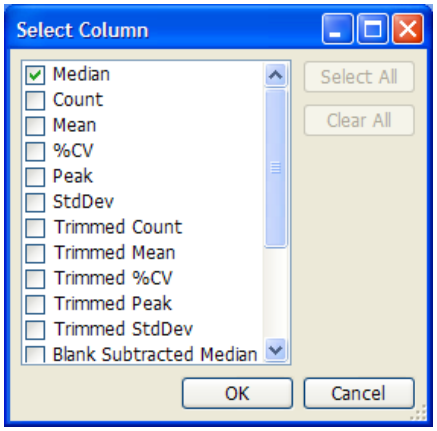
Menu	Description
Select Column	Opens the *Select Column* dialog box. Select one column of data to display. See ***Selecting a Column to Display*** on page 2- 21.

Menu	Description
Set Title	Opens the *Set Chart Title* dialog box. Enter a new title for the scatter plot and click **OK**. See ***Setting a Title for a Plot or Chart*** on page 2-29.
Set Axes Ranges	Opens the *Set Axes Ranges* dialog box. Specify the display range of the x and y axes. For scatter plots, you can set either axis, or both axes. See ***Setting the Axes Ranges (Scatter Plot or Bar Chart)***.
Set Axes Numeric Type	Opens the *Set Axes Numeric Type* dialog box. Here you can specify the numeric type for either axis as: **Linear** - is the default - or - **Logarithmic -** select this if a wide spread in values make it difficult to view all of the data on a linear scale. Choose **common**, **natural**, or **binary**, depending on your data and preferences. See ***Changing the Axes Numeric Type***.
Zoom Full	Click to return to a full display of the chart after changing the axes ranges. This menu selection is dimmed and unavailable if the chart is already at full display.
Hide Lines/Show Lines	Click to hide the lines if they are showing, or show the lines if they are hidden. The red line represents y=x, allowing a quick visual determination of which axis has the higher value. If a data point is above the red line, y > x. If a data point if below the red line, y < x. The blue lines are n-fold lines; that is, y = n * x or y = (1/n) * x. In this release of the analysis software, n = 2. If a data point is above the top blue line, y > 2 * x. If a data point is below the bottom blue line, y < 1/2 * x.

2.6.2.3 Selecting a Column to Display

To select a column to display on the scatter plot

1. From the **View** menu, select **Scatter Plot Options, Select Column.**

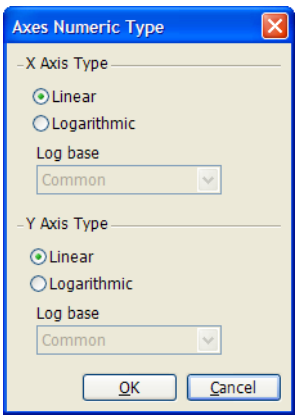


Select the column to view. For scatter plots, only one column at a time can be selected. The **Select All** and **Clear All** buttons are dimmed and unavailable.

2.6.2.4 Changing the Axes Numeric Type

To change the axes numeric type

1. With the scatter plot open, right-click in the plot to open the menu and select **Set Axes Numeric Type**. The *Axes Numeric Type* dialog box opens.



2. For one or both axes, select the axis type: **Linear** or **Logarithmic**.

3. If Logarithmic is selected, select a log base from the drop-down list: Common, Natural, or Binary.

4. Click **OK** to save the changes.

2.6.3 The Bar Chart

The bar chart displays the values across wells for the user-selected analytes, or values for all analytes in a single well. The configurable chart allows selecting which columns of data to display, and which analyte or location to view.

Select one, several, or all columns for the display. The legend on the right displays the color that represents each column.

The title of the chart displays the name of the selected analyte or well location, or a title that's been assigned under the Bar Chart Options described in this section. The bar chart can be printed or saved. See ***Saving the Analysis Results***.

Figure 2-11 is an example of a bar chart. Hold the cursor over any data point to display information.

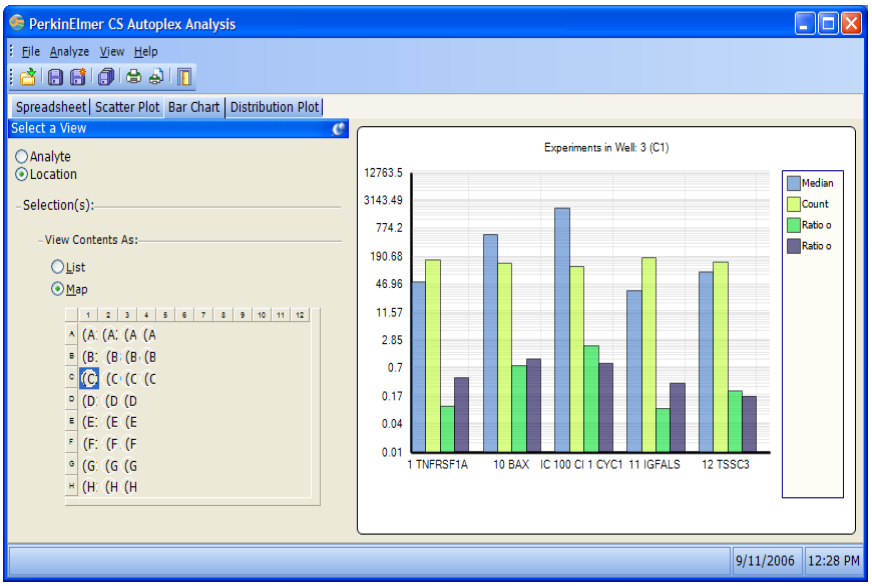


Figure 2–11 Example of a Bar Chart

To change the bar chart view

1. Click **Select a View** on the left-hand side of the bar chart. The *Select a View* toolbox opens over the chart.

2. Select **Analyte** as the view and select an analyte from the list;
   or,
   Select **Location** as the view, and select a location from the list or map.
   The toolbox automatically auto-hides when you click anywhere on the bar chart.

Note: To keep the toolbox open and display the entire chart and toolbox side by side, click the pushpin on the toolbox. Click the pushpin again to return to Auto Hide.

2.6.3.1 Display Options for the Bar Chart

To configure the display

1. On the **View** menu, select **Bar Chart Options**
   Or,
   right-click anywhere on the bar chart to open the options menu.

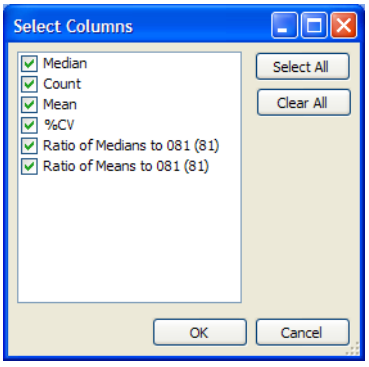
2. From the options menu, select the option you want to change. The options are described in the following table.

Menu	Description
Select Columns	Opens the *Select Columns* dialog box. Select one, several, or all columns of data to display. See ***Selecting Columns for Bar Chart Display*** on page 2- 24.
Set Title	Opens the *Set Chart Title* dialog box. Enter a new title for the bar chart and click **OK**.
Set Axes Ranges	Opens the *Set Axes Ranges* dialog box. Select this option to specify the display range of the x and y axis, or to specify a scroll bar for one or both axes. See ***Setting the Axes Ranges (Scatter Plot or Bar Chart)***.
Set Y Axis Numeric Type	Opens the *Y Axis Numeric Type* dialog box. Here you can specify the numeric type for the Y axis as: **Linear** (default) - or - **Logarithmic,** if a wide spread in values make it difficult to view all of the data on a linear scale. Choose **common**, **natural**, or **binary**. See ***Changing the Y Axis Numeric Type*** on page 2- 25.
Zoom Full	Click to return to a full display of the chart. This menu selection is dimmed and unavailable if the chart is already at full display.

2.6.3.2 Selecting Columns for Bar Chart Display

To select column(s) to display on the bar chart

1. From the **View** menu, select **Bar Chart Options, Select Columns.**



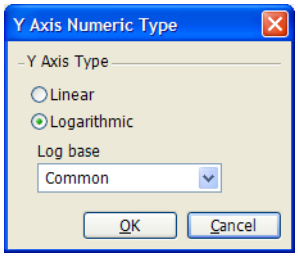
2. Check the box for each column to display. Any number of columns can be selected for bar chart display.

   - Click **Select All** to display all columns, click **Clear All** to clear all checkboxes.

2.6.3.3 Changing the Y Axis Numeric Type

To change the Y axis numeric type

1. With the scatter plot open, right-click in the plot to open the menu and select **Set Y Axis Numeric Type**. The *Y Axis Numeric Type* dialog box opens.



2. Select the axis type: Linear or Logarithmic.

3. If Logarithmic is selected, select a log base from the drop-down list: Common, Natural, or Binary.

4. Click **OK** to save the changes.

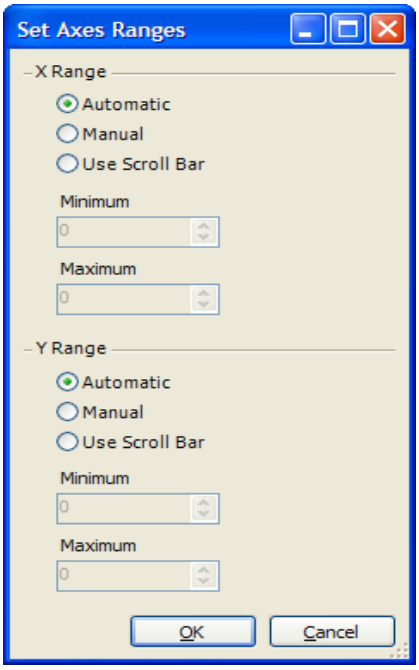
2.6.4 Setting the Axes Ranges (Scatter Plot or Bar Chart)

You can set either or both axes ranges for the scatter plot and bar chart to change the data display, or select scroll bars to quickly view data by expanding or collapsing an axis, or view data by scrolling along either axis. In addition, you can choose either linear or logarithmic as the numeric type for the scatter plot axes, or the bar chart y axis.

To set the axes ranges

1. Click the tab for the scatter plot or bar chart.

2. Right-click in the plot to open the menu and select **Set Axes Ranges**. The *Set Axes Ranges* dialog box opens.



3. Refer to the following table and set the ranges. Click **OK**.

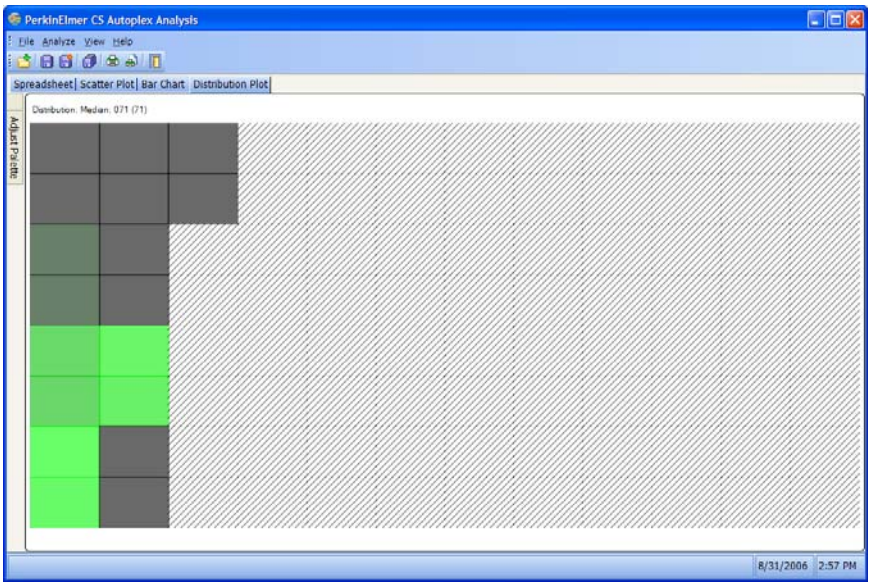
Item	Description
Y Range or X Range	Each axis range has the following range options:
Automatic	Select to have the PerkinElmer CS Autoplex Analysis software automatically set the range for the axis.
Manual	Select to specify the range manually, enter the values: **Minimum**: Enter a value for the range minimum (it must be lower than the maximum range value). **Maximum**: Enter a value for the range maximum.
Use Scroll Bars	Select to add scroll bars that can expand or compress, and lets you scroll through the axis to better view the data. Available for bar charts and scatter plots.

2.6.5 Distribution Plot

The Distribution Plot shows the distribution of values for the selected analyte across all wells. The configurable plot allows selecting which column of data to display, which analyte to view, and provides a palette adjustment tool for screening the results viewed, based on high and/or low thresholds.

Note: A distribution plot is available only if the Export Location Label Style selected in the Luminex IS data export options is **Plate Location** or **Both**. See ***Input Data for the Analysis*** on page 2- 1.

Hold the cursor over any block to see information about the intensities of the selected analyte.



The distribution plot, as displayed, can be printed or saved. See ***Saving the Analysis Results***.

2.6.5.1 Display Options for the Distribution Plot

The distribution plot is configurable so that any column of data can be selected for plotting, and any analyte can be selected for viewing. The ratiometric data is displayed using a green/black/red palette, as follows:

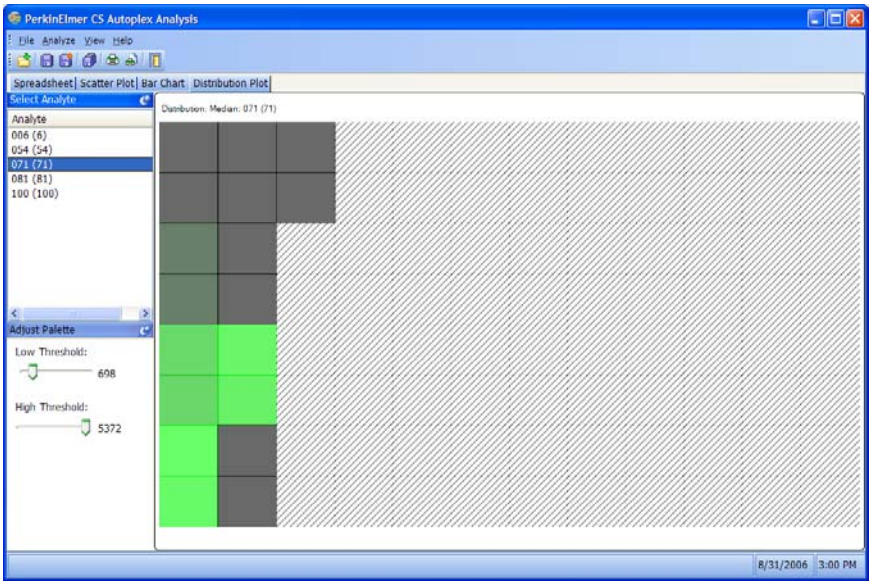
- Green - Indicates a value less than one (<1)
- Black - Indicates a value of approximately one (1)
- Red - Indicates a value greater than one (>1)

Non-ratiometric data is displayed using a green/black palette as follows:

- Black - indicates low values
- Bright green - indicates high values

To select a different view

1. Click **Select Analyte** on the left-hand side of the *Distribution Plot* tab. The *Select Analyte* toolbox opens over the plot.



2. Select an analyte from the list. The toolbox auto-hides when you click anywhere on the plot.

Note: To keep the *Select Analyte* toolbox open and display the entire plot and toolbox side by side, click the pushpin on the toolbox. Click the pushpin again to return to Auto-Hide.

To adjust the palette

1. Click Adjust Palette on the left-hand side of the *Distribution Plot* tab. The *Adjust Palette* toolbox opens over the plot.

Note: To keep the *Adjust Palette* toolbox open and display the entire plot and toolbox side by side, click the pushpin on the toolbox. Click the pushpin again to return to Auto-Hide.

2. Under **Low Threshold,** move the slider to the right to raise the low-threshold value or move the slider to the left to lower the low-threshold value.

3. Under **High Threshold**, move the slider to the left to lower the high-threshold value or move the slider to the right to raise the high-threshold value.

Using the two sliders, you can effectively narrow the range of values being viewed to those within a certain threshold range.

To configure the display

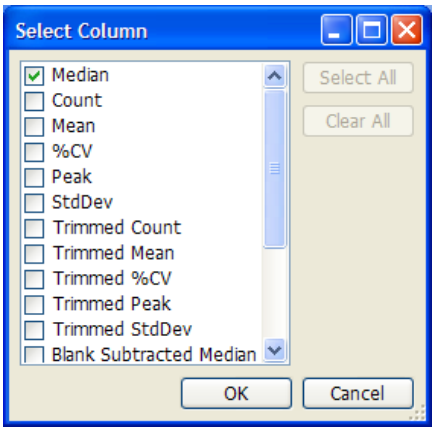
1. Right-click anywhere on the distribution plot,
   Or,
   on the **View** menu, select **Distribution Plot Options**.

2. From the options menu, select the option you want to change. The options are described in the following table.

Menu	Description
Select Column	Opens the *Select Column* dialog box. Select one column of data to display.
Set Title	Opens the *Set Chart Title* dialog box. Enter a new title for the distribution plot and click **OK**.

To select a column to display on the distribution plot

1. From the **View** menu, select **Distribution Plot Options, Select Column**.



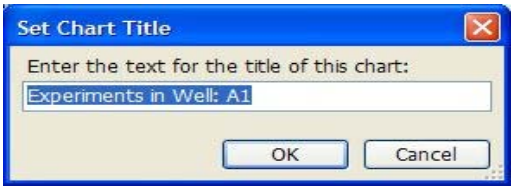
2. Select the column to view. For distribution plots, only one column at a time can be selected. The **Select All** and **Clear All** buttons are dimmed and unavailable.

2.6.6 Setting a Title for a Plot or Chart

Before printing or saving a particular view of a chart, you may want to change the title to something more meaningful.

To set a title for a plot or chart view

1. Right-click anywhere on the plot or chart. The *Set Chart Title* dialog box opens.



2. Type a name for the chart and click **OK**.

2.7 Saving the Analysis Results

You can save the spreadsheet table as a .csv file, and save a plot or chart as a BMP, GIF, PNG, JPEG, or TIF file. Any spreadsheet or plot can be saved individually. If you use the **Save all** command, the current view of all open plots can be saved with the spreadsheet.

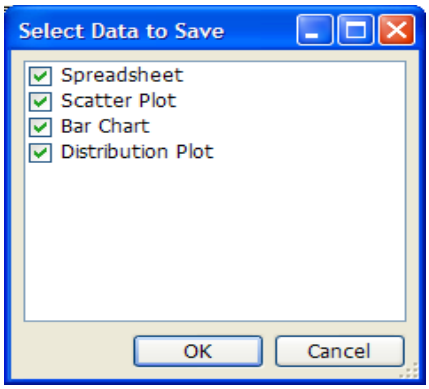
Note: To save a particular view of a scatter plot, bar graph, or distribution plot, save it individually while it is displayed using the **Save** or **Save as** command.

If you select **Close** before saving, the PerkinElmer CS Autoplex Analysis prompts you to save.

2.7.1 Using the *Save All* Command

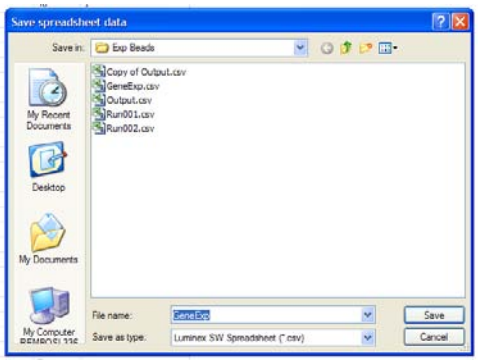
To save all of results

1. On the **File** menu, click **Save All**. The *Select Data to Save* dialog box opens.
2. Check each results format that you want to save, and click **OK**.

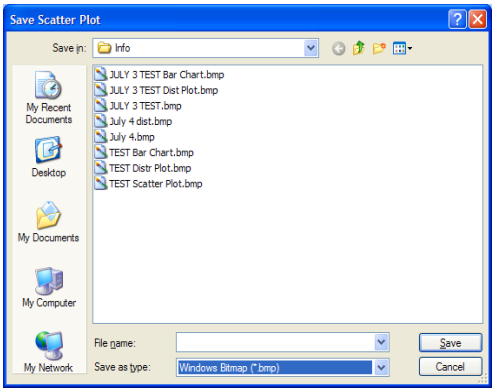


A *Save* dialog box opens for each tab as follows:

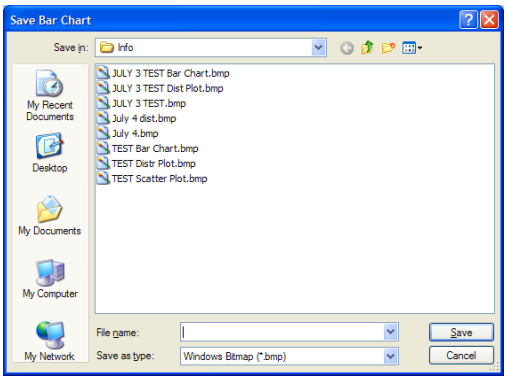
3. In the *Save Spreadsheet* dialog box, enter a name and click **Save**.



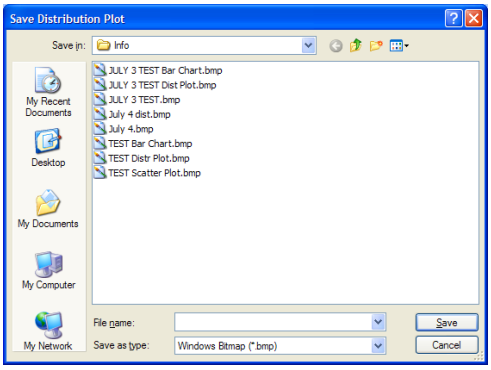
4. In the *Save Scatter Plot* dialog box, enter a name, select the file type, and click **Save**.



5. In the *Save Bar Chart* dialog box, type a name, select the file type, and click **Save**.



6. In the *Save Distribution Plot* dialog box, type a name, select the file type, and click **Save**.

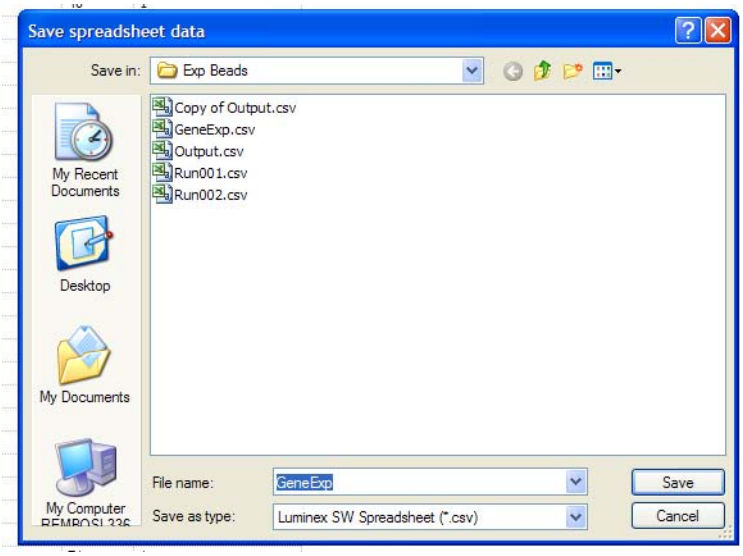


All of the results are saved and can be re-opened for review.Saving the Spreadsheet

2.7.2 Saving the Spreadsheet

To save the spreadsheet results

1. On the **File** menu, select **Save** or **Save as**. The *Save spreadsheet data* dialog box opens.



2. Type a name for the spreadsheet in the **File name** box, and click **Save**.

2.7.3 Saving an a Plot or Chart

To save a scatter plot, bar chart, or distribution plot

1. Display the plot or chart view that you want to save,
2. On the **File** menu, select **Save** or **Save as**. If the view has already been saved, it is saved again, overwriting the previous file. If the view has not been saved, the *Save as* dialog box opens.
3. Type a name in the **Filename** field, and select a file type (.bmp, .gif, .jeg, .tif, or .png) in the **Save as type** file.
4. Click **Save**.

2.7.4 Printing a Spreadsheet or Plot

You can print the current spreadsheet or any of the plots with or without saving them.

To print a spreadsheet or plot

1. Click the tab for the spreadsheet, or for any plot.
2. On the **File** menu, select **Print** (or select **Print Preview** first to see how it looks).
3. In the **Print** window, specify the printer and click **OK**.

2.8 Opening Saved Results

To open saved analysis results

1. On the **File** menu, click **Open**. The *Open* dialog box appears.
2. Enter the name of the results file in the **File name** field.
3. In the **Files of type**, select the PerkinElmer CS Autoplex Analysis Software Output (*.csv).

Note: The results files will not open unless *PerkinElmer CS Autoplex Analysis Software Output* is selected as the type of file.

4. Click **Open**. The spreadsheet table opens, and any plots are regenerated with default settings.

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Installing the PerkinElmer CS Autoplex Analysis Software

Appendix A

Appendix Summary

A.1 Overview

The PerkinElmer CS Autoplex Analysis Software is provided on a distribution web site, at:

www.perkinelmer.com/csautoplex

A.2 Installing the Software

The software includes an InstallShield Wizard for installing the software.

To install the software

- Run the setup.exe file to start the software installation.

The InstallShield Wizard installs the software on your computer..

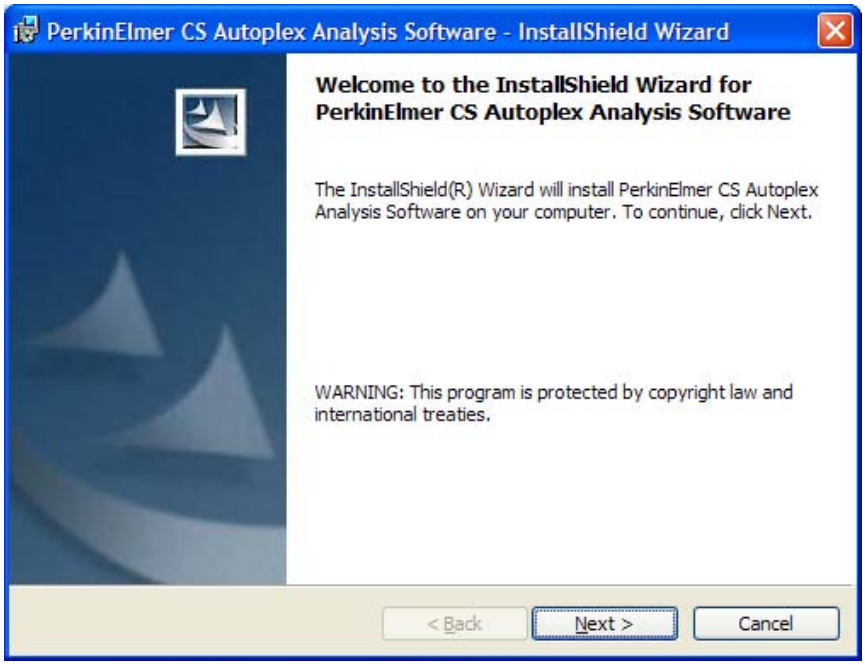

Figure A-1 The First InstallShield Window

Follow the prompts to install the software.

Adding Blanks and Controls Information

Appendix B

Appendix Summary

B.1 Overview

Information for blank samples and normalization controls should already be included with the data exported from the Luminex software. The PerkinElmer CS Autoplex Analysis software uses the sample names beginning with the word “Blank” to perform blank subtraction and test names beginning with “IC” or “EC” (for internal or external controls) to perform normalization.

If the information is not in the data file, it can be added by editing the data file in one of two ways:

- The data file can be modified using Microsoft Notepad, as described in this appendix.
- The data file can be modified using the spreadsheet within the PerkinElmer CS Autoplex Analysis Software (See ***Adding Blanks or Controls Information after Acquisition*** on page 2- 11.

Note: Do not edit the data file in Microsoft Excel. Excel modifies the data formatting when saving the file, making the file unusable by the PerkinElmer CS Autoplex Analysis software.

B.2 Adding Blanks Information using Notepad

The batch data may be replayed to include the keyword that indicates a blank sample. However, it is easier to edit the file by modifying the sample name strings for each blank. Only the Median data rows need to be modified.

To add blanks information

1. Open the original Luminex IS file in Notepad.

2. Find each blank well location and modify the Sample name to begin with the word "Blank." In the following example, the well location "C4" was located in the file, and the sample name changed to blank.

"C4","Blank
2","5.5","10","34.5","0","4","11.5","3.5","7","11","0","13","1","0","8.5","0","8","17","7","4","0.5","7","17","0","7.5","12","3","6.5","11","8.5","4","7","3","0","15","7","11","14","10","11.5","8","2.5","13.5","6","3","24.5","9","7","10","13","8.5","1","4","8","9","20","10","4","16","10","0","10","11","23","3.5","11","13.5","8.5","3","19","18.5","18","15","11","12","16","16","9","24","9","11","15.5","15","13.5","10.5","25","15.5","20","19","19","3","22.5","20","25","25","28.5","25","32","25","29","8271",""

3. Save and close the file. The file is ready to re-open in the CS Autoplex Analysis software.

B.3 Adding Controls Information Using Notepad

The batch data may be replayed to include the control keywords ("IC" and "EC"), but it is easier to edit the file manually.

To add control information

1. Open the original Luminex IS file in Notepad.

2. All data type data sets need to be modified; the easiest way to do this is using "replace all."
On the Notepad **Edit** menu, select **Replace**. In the *Replace* dialog box, specify the string to find, the string to replace it, and click **Replace All**. Use a unique original string so that the replacement works correctly.

3. Save and close the file. The file is ready to re-open in the CS Autoplex Analysis software.

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Glossary

Analyte	A substance that is detected through assay analysis. Each test or bead set tests for a specific analyte.
Analyte ID	A short text name for the analyte, or bead region.
Analyte Name	A text description for the analyte or bead region.
Analyzer	The Luminex 200 (100 IS?) analyzer used to create xMAP files.
Bead	Refers to an xMAP microsphere. See microsphere.
bead region	Refers to an xMAP microsphere. See microsphere.
bead set	A set of xMAP microspheres that have a uniquely identifiable ratio of two classification dyes. The unique ratio is identified by a unique spectral (?) address.
control beads	Used to verify standards.
Replicates	Multiple beads? with the same analyte in the same concentration, having the same ID and name.
Replicate Samples	The data set produced using the same sample multiple times (in the same bead region?) .
Luminex xMAP microsphere set	Luminex multi-analyte microspheres containing a unique mixture of two distinctly colored fluorochromes to distinguish them from other multi-analyte microspheres.
microspheres	Polystyrene spheres with a diameter in the micrometer range. Also called beads.
multi-analyte	Several assays or tests performed simultaneously in the same reaction containers.
qualitative	Pertaining to calculations that determine the absence or presence of an analyte.
quantitative	Pertaining to calculations that determine the precise numerical measurement of an analyte.
Sample	The mixture of assay components (microspheres, reporter, patient diluent) that are analyzed.
Sample Name	A text description assigned for a sample.

Standards microspheres, assay	Assay standards are substances of known concentrations used to derive a standard curve with which unknown samples and controls are compared to determine their concentration or quantity.
xMAP	See Luminex xMAP microsphere set.

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